Original Article

Thai Journal of Pharmaceutical Sciences

Comparative evaluation of the effects of n-hexane, chloroform, and methanol fractions of *Ricinus communis* in carbon tetrachloride-induced hepatotoxic rats

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Received: Jul 09, 2019
Accepted: Dec 08, 2019
Published: Jan 02, 2020

ABSTRACT

Background: *Ricinus communis* is widely used among the Nigerian populace in ethnomedicine for the treatment of liver diseases. **Aim of the Study:** A comparative study was carried out to evaluate the hepatoprotective activity of its n-hexane, chloroform, and methanol fractions using carbon tetrachloride-(CCL_4)-induced hepatotoxicity model. **Materials and Methods:** Thirty Wistar rats were randomized as follows: Negative control Group 1 received Tween 80 and positive Group 2 received CCL_4 only. Treatment Groups (3, 4, and 5) received CCl_4 + N-hexane, chloroform, and methanol fractions, 100 mg/kg, respectively. Standard reference, Group 6, received CCl_4 + Silymarin, 100 mg/kg. The animals were treated for 7 consecutive days and hepatotoxicity was induced in Groups 1, 2, 3, 4, and 6 by a single oral administration of CCL_4 (2 mL/kg). Thereafter, blood samples were collected for assay of hematological parameters and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]). **Results:** There were significant decrease ($P < 0.05$) in serum AST/ALT levels of animals treated with chloroform fraction ($50.00 \pm 6.01\,\mu$/L/$21.40 \pm 0.98\,\mu$/L) and methanol fraction ($51.25 \pm 2.88\,\mu$/L/$24.80 \pm 1.52\,\mu$/L). The chloroform fraction produced a better improvement in hematological parameters when compared with other treatment groups. Photomicrograph showed near-normal structural liver integrity of the rats administered chloroform fraction. **Conclusions:** This study validates the usefulness of *R. communis* in the treatment of hematological disorders in patients with hepatic disorders. The chloroform fraction could be a promising fraction for further investigation and characterization of hepatoprotective compound(s).

Keywords: Carbon tetrachloride, hematology, hepatoprotection, *Ricinus communis*, rodents

INTRODUCTION

The liver is a vital organ involved in extramedullary hematopoiesis, the synthesis of transferrin (a carrier protein that transports iron for erythropoiesis), coagulation factors, storage of Vitamin B12, folic acid, and iron as well as the destruction and removal of red cells.[1,2] Although the liver has a high regenerative capacity, the numerous roles of the organ, especially detoxification and metabolism, can overwhelm its recovery from injury.

Chemical-induced hepatic damage is becoming overwhelming due to exposure of subjects to environmental and industrial chemicals as well as food and drug poisoning. Furthermore, its metabolic function puts the liver at a strategic disadvantage of injury, especially of toxicants triggered by metabolism through hepatic enzymes. Compromised liver function following hepatic injury could render the body system prone to serious health complications as a result of immune suppression.[3] Chronic liver diseases, in most cases, are associated with hematological disorders. Anemia has been shown to occur in 75% of patients with chronic liver diseases.[4] Studies have shown that carbon tetrachloride (CCL_4) toxicity caused anemia as well as impairment in red blood cell (RBC) production.[3,5]

In the management of liver disorders, no ideal synthetic hepatoprotective agents have been discovered because

some of them have limitations in their use. In light of this, drug discovery/development studies including alternative approaches in the form of herbal medicine are being pursued. To this end, a number of medicinal plants such as *Pueraria lobate* and *Withania somnifera* and isolated molecules such as silymarin have been reported to possess hepatoprotective activity.[6,7]

Ricinus communis, popularly known as castor oil plant, belongs to the family Euphorbiaceae, an evergreen shrub growing up to 5 m tall that is widespread in tropical areas. A recent review by Manoj[8] revealed that it possesses several medicinal values which have been validated through pharmacological screening. Among these include antiulcer,[9] antioxidant,[10] antidiabetic,[11] as well as hepatoprotective activities.[12-14] Phytochemical evaluation of *R. communis* revealed the presence of anthraquinones, alkaloids, phobatannin, flavonoids, saponins, steroids, and glycosides.[15]

In an attempt to identify the most promising fractions which could be of usefulness in drug development, the present study evaluated the effect of n-hexane, methanol, and chloroform fractions of *R. communis* using hematological and liver enzymes as biomarkers of hepatic function in CCL_4 model in rats.

MATERIALS AND METHODS

Preparation of Plant Fraction

Fresh leaf of *R. communis* was collected from the wild in Delta State, Nigeria. The plant was authenticated with a voucher deposit (FHI 108864) at the Forestry Research Institute Nigeria. The leaf of *R. communis* was cleaned with water, dried at room temperature and pulverized. A total of 1.8 kg of the powdered leaves was cold-extracted serially with a known volume of n-hexane, chloroform, and methanol, consecutively. At each stage, the filtrate was concentrated with a rotary evaporator to obtain the n-hexane, chloroform, and methanol fractions, respectively. The percentage yield for each fraction was calculated. The fractions were reconstituted in Tween-80 before the administration to the laboratory animals; the concentrated fraction could not dissolve in water. The doses of the fractions were determined following a pilot study and literature search.[16]

Animals

A total of 30 healthy albino rats used for this study were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria. They were given access to rat chow and water *ad libitum*. Animals were allowed to acclimatize for 14 days before the commencement of the study. All experimental procedures were consistent with the NIH guidelines for the use and care of laboratory animals, as approved by the Ethics Committee of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria (FBMS/234/8102).

Induction of Hepatotoxicity

Experimental animals were randomly allotted into six groups of five animals each. After fasting the animals for 12 h, hepatotoxicity was induced by a single oral administration of CCl_4 (2 mL/kg) to the experimental animals.[17] N-hexane, chloroform, and methanol fractions of *R. communis* (100 mg/kg) were administered to Groups 1, 2, and 3, respectively. Group 4 received Silymarin (100 mg/kg). Groups 5 and 6 served as CCl_4 and normal control (Tween 80), respectively. Treatment was done orally, once daily and it lasted 7 days.

Sample Collection

The animals were fasted for 12 h after the 7 days treatment. Blood samples were collected from the rats for hematological and liver biomarkers assay. The rats were then sacrificed and the liver harvested for histomorphological examinations.

Assay of Hematological and Liver Biomarkers

Hematological parameters, hemoglobin, hematocrit packed cell volume (PCV), RBC, platelet counts, white blood cell (WBC), and differentials (neutrophils and lymphocytes) were determined using the method described by Baker *et al*.[17] Liver enzymes, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using the method of Reitman and Frankel.[18]

Data Analysis

Results are presented as mean ± standard deviation. Significant difference among groups was determined by one-way analysis of variance, using Statistical Package for the Social Science (SPSS-20, for windows). $P < 0.05$ was considered to be statistically significant.

RESULTS

The percentage yield for each of the fractions is presented as follows:

- N-hexane: 60/1800 × 100/1 = 3.33%
- Chloroform: 67/1800 × 100/1 = 3.72%
- Methanol: 76/1800 × 100/1 = 4.22%.

Results [Table 1] showed that there was a significant decrease ($P < 0.05$) in serum AST level of animals that were treated with chloroform fraction (50.00 ± 6.01 μ/L), methanol fraction (51.25 ± 2.88 μ/L), and silymarin (49.60 ± 8.38 μ/L) when compared with AST level of animals in positive control (75.23 ± 5.06 μ/L) [Table 1]. In the same vein, serum ALT levels were observed to be significantly ($P < 0.05$) reduced following treatment with chloroform fraction (21.40 ± 0.98 μ/L), methanol fraction (24.80 ± 1.52 μ/L), and silymarin (22.62 ± 0.43 μ/L) when compared with ALT level of animals in positive control (40.33 ± 1.50 μ/L). The levels of the liver enzymes were similar in the animals administered standard agent (silymarin) and chloroform/methanol fractions. The same trend was also observed when the test groups administered chloroform/methanol fractions were compared with the negative control groups (normal animals administered Tween 80 only).

It was observed that administration of the fractions and silymarin caused a significant ($P < 0.05$) increase in PCV, platelet, WBC, neutrophils, and lymphocytes levels

when compared with the untreated group (positive control) [Table 2]. A similar trend was also observed in RBC and hemoglobin, except for methanol fraction which did not produce any observable difference in PCV as well as n-hexane fraction which did not produce any observable difference in hemoglobin when compared with the negative control. Overall, the chloroform fraction produced a better improvement in hematological parameters when compared with other treatment groups.

Photomicrographs of sections of the liver of the experimental animals are shown in Figure 1a-f. Worthy of mention is near-normal structural integrity of sections of the liver of the rats administered silymarin [Figure 1c] and chloroform fraction [Figure 1f] of the test agents (*R. communis*) compared with that of the rats in the normal control group [Figure 1a] administered the vehicle (Tween-80). Comparatively, the protective effect of the aforementioned fractions is also visible against the effect of the hepatotoxic agents (CCL_4), namely, multiple foci of hepatic necrosis and fatty infiltration and severe portal congestion, in the group administered CCL_4 only [Figure 1b].

DISCUSSION

The present study evaluated the hepatoprotective potentials of three fractions of *R. communis* leaf utilizing CCL_4 model of hepatotoxicity in rats. The CCL_4-induced hepatic damage is an established model of investigating the hepatoprotective effects of drugs undergoing screening. The substance has been known to produce toxic effects on the liver through its conversion to a trichloromethyl free radical by the hepatic CYP_{450} microsomal enzymes. The chain of reactive free radicals generated causes lipid peroxidation, damage of proteins, and other cell components.[19] This is also characterized by an elevation in the liver biomarkers, ALT, and AST, among others.[3] ALT and AST are largely used as biomarkers of xenobiotic-induced liver damage, which is characterized by elevated AST and ALT level as a result of a disruption in the integrity of hepatocytes plasma membrane and loss of their content into the blood.[20] The observed increase in ALT and AST, in the present study, is similar to the findings of Saba *et al*.[3] From this study, a significant elevation in ALT and AST levels observed following the administration of CCL_4[21] was reduced after treatment with chloroform and methanol fractions, suggesting that the constituents in these fractions could be hepatoprotective. This finding is supported by the previous studies on the hepatoprotective effect of *R. communis* due to its ability to inhibit lipid peroxidation in the liver as well as the activities of elevated liver enzymes.[13,14] The protective effect of the chloroform fraction was further buttressed by the near-normal structural integrity of the liver tissues of the rats administered this test agent. This is in line with the findings of Shukla *et al*.[13] who reported increase viability of hepatocyte and near-normal restoration of enzymatic levels.

Administration of CCL_4 reported by a previous study[3] to cause derangement of hematological parameters was confirmed by the observation in the present study. Saba *et al*. reported deranged hematological parameters culminating in anemia.[3] In this study, the administration of *R. communis* produced a significant reduction in the effects of CCL_4 on hematological parameters. These effects may be attributed to the protective and reparative effect of the bioactive components of the fractions on the liver thereby preserving its functions which include the synthesis of transferrin and coagulation factors. A better effect produced by the chloroform fraction when compared with other groups could be attributed

Table 1: Effect of fractions of *Ricinus communis* on AST and ALT levels of CCL_4 induced hepatotoxicity in rats

Group	Treatment	Serum AST (μ/L)	Serum ALT (μ/L)
Group 1	Negative control (Tween 80)	48.37±1.67[a]	20.62±0.43[a]
Group 2	Positive control (CCL_4 only)	75.23±5.06[b,c]	40.33±1.50[b,c]
Group 3	CCL_4+n-hexane fraction	71.19±7.78[b,c]	32.40±1.49[b,c]
Group 4	CCL_4+chloroform fraction	50.00±6.01[a]	21.40±0.98[a]
Group 5	CCL_4+methanol fraction	51.25±2.88[a]	24.80±1.52[a]
Group 6	Standard control (CCL_4+silymarin)	49.60±8.38[a]	22.62±0.43[a]

Values are expressed as mean±standard deviation, n=5. [a,b,c]P<0.05: Statistically significantly different from CCL_4 control (positive control), Tween 80 (negative control), and silymarin (standard control), respectively. CCL_4: Carbon tetrachloride, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 2: Effect of fraction s of *Ricinus communis* on hematological parameters of carbon tetrachloride induced hepatotoxic rats

Group	Packed cell volume (%)	Hemoglobin (g/dl)	Red blood cell×10^6/μL	Platelet×10^3/μL	White blood cell×10^3/μL	Neutrophils (%)	Lymphocyte (%)
Group 1	47.20±3.96*	14.26±1.19*	5.64±0.56*	308.40±20.14*	8.84±2.69*	12.40±2.07*	77.60±6.47*
Group 2	29.17±0.99[b,c]	11.74±0.5[b,c]	4.49±0.19[b,c]	118.40±14.4[b,c]	4.68±0.72[b,c]	3.68±0.09[b,c]	50.70±0.30[b,c]
Group 3	42.25±0.50*	12.78±0.86*	4.58±0.56[b,c]	210.50±14.20*	6.50±0.79*	10.25±0.50*	79.75±0.50*
Group 4	46.67±2.31*	14.50±0.69*	5.27±0.25*	294.33±12.50*	7.63±1.53*	12.00±1.00*	82.00±7.00*
Group 5	42.33±1.53*	11.80±1.48[b,c]	5.07±0.21*	245.33±21.39*	7.20±1.37*	11.67±2.89*	70.00±8.66*
Group 6	46.80±2.68*	14.14±0.80*	5.50±0.64*	291.80±20.84*	7.78±3.14*	11.80±2.28*	80.20±2.28*

Values are expressed as mean±standard deviation, n=5. *, [b,c]P<0.05: Statistically significantly different from Group 2 (positive control), Group 1 (negative control), and Group 6 (standard)

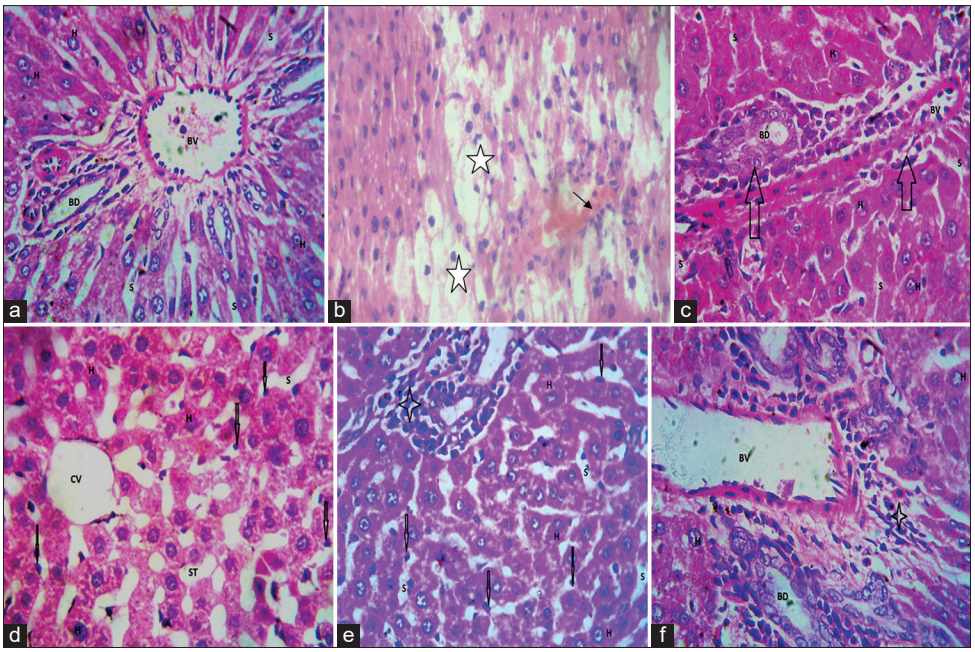

Figure 1: ×400 H and E staining - (a) Normal control: Photomicrograph shows the hepatic portal regions composed of blood vessel (BV), bile duct (BD). Plates of hepatocytes (H) separated by sinusoids (S) that appear essentially unremarkable. (b) Carbon tetrachloride (CCL_4) control: Photomicrograph shows the liver of rat administered CCL_4 only showing multiple foci of hepatic necrosis and fatty infiltration (stars). Severe portal congestion (arrowed) (c) CCL_4+silymarin (100 mg/kg): The section shows the hepatic portal region (arrow) with mild periportal inflammatory cell infiltration. The BV, BD, H, and sinusoids appear normal. (d) CCL_4+methanol fraction (100 mg/kg): Photomicrograph shows a section with marked activation of hepatic macrophage (arrow) within the sinusoids (s) also seem mild steatosis (ST) (e) CCL_4+n-hexane fraction (100 mg/kg): Photomicrograph shows a section with marked activation of hepatic macrophage (arrow) within the sinusoids (s) and moderate periportal hepatitis (f) CCL_4+chloroform fraction (100 mg/kg): Photomicrograph shows a section of the hepatic tissue composed portal region (blood vessel BV and bile duct BD) section also show mild periportal hepatitis (star)

to the possibility of chloroform as a solvent to fraction more of the phytochemical compound(s) responsible for the effect on the deranged hematological parameters. It could be inferred, therefore, that the active component was isolated by and located in the chloroform fraction. Studies have shown that chloroform has the ability to fraction some secondary metabolites with biological activity.[22,23] Going by its being a mid-polar organic solvent, chloroform characteristically dissolves both non-polar and some moderately polar molecules. In light of this, the active hepatoprotective compounds in *R. communis* may be non-polar to moderate polarity.

However, it has been reported that secondary metabolites of medicinal plants are responsible for their biological activities.[14] Thus, the presence of bioactive phytochemicals such as flavonoids and tannins in *R. communis* may also be responsible for the regenerative and reparative capacity of the rat liver during hepatic injury caused by CCL_4.

CONCLUSIONS

This study demonstrates that the chloroform fraction of *R. communis* improved CCL_4 altered liver and hematological parameters. This finding provides preliminary data which may support the use of *R. communis* in the treatment of hematological disorders in patients with hepatic damage or impairment. Further studies to elucidate the bioactive constituents responsible for the hepatoprotective activity of the chloroform fraction are hereby recommended. The active metabolites of *R. communis* responsible for maintaining the structural and functional integrity of the liver may be located in the chloroform fraction.

REFERENCES

1. Birbrair A, Frenette PS. Niche heterogeneity in the bone marrow. Ann N Y Acad Sci 2016;1370:82-96.
2. Sherwood RA, Bomford A. Assessment of hepatic function and investigation of jaundice. In: Marshall WJ, Lapsley M, Day AP, Ayling RM, editors. Clinical Biochemistry. London: Churchill Livingstone, Elsevier; 2014. p. 231-49.
3. Saba AB, Oyagbemi AA, Azeez OI. Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf fraction of *Cnidoscolus aconitifolius* in rats. Niger J Physiol Sci 2010;25:139-47.
4. McHutchison JG, Manns MP, Longo DL. Definition and management of anemia in patients infected with hepatitis C virus. Liver Int 2006;26:389-98.
5. Elmubarak S, Özsoy N. Histoprotective effect of Vitamin D against carbon tetrachloride nephrotoxicity in rats. Hum Exp Toxicol 2016;35:713-23.
6. Tang CC, Huang HP, Lee YJ, Tang YH, Wang CJ. Hepatoprotective effect of mulberry water fractions on ethanol-induced liver injury via anti-inflammation and inhibition of lipogenesis in C57BL/6J mice. Food Chem Toxicol 2013;62:786-96.
7. Wang QK, Song YF, He YH, Ren DD, Kow F, Qiao ZY, *et al*. Structural characterisation of algae Costaria costata fucoidan and its effects on CCl4-induced liver injury. Carbohydr Polym 2014;107:247-54.
8. Manoj K. A Review on phytochemical constituents and pharmacological activities of *Ricinus communis* L. Plant Int J Pharmacogn Phytochem Res 2017;9:466-72.

9. Rachhadiya RM, Kabra MP, Shete RV. Evaluation of antiulcer activity of castor oil in rats. Int J Res Ayurveda Pharm 2011;2:1349-53.
10. Kumar GM, Sharma PK, Ansari SH. *In-vitro* antioxidant activity of the successive fractions of *Ricinus communis* leaves. Int J Plant Sci 2006;1:229-31.
11. Gad-Elkareem MA, Abdelgadir EH, Badawy OM, Kadri A. Potential antidiabetic effect of ethanolic and aqueous-ethanolic extracts of *Ricinus communis* leaves on streptozotocin-induced diabetes in rats. PeerJ 2019;7:e6441.
12. Natu MV, Agarwal S, Agarwal SL, Agarwal S. Protective effect of *Ricinus communis* leaves in experimental liver injury. Indian J Pharmacol 1977;9:265-8.
13. Shukla B, Visen PK, Patnaik GK, Kapoor NK, Dhawan BN. Hepatoprotective effect of an active constituent isolated from the leaves of *Ricinus communis* Linn. Drug Dev Res 1992;26:183-93.
14. Pappithi RB, Cherukupalle B, Gandham S, Chintha VR, Wudayagiri R. Hepatoprotective role of *Ricinus communis* leaf extract against d-galactosamine induced acute hepatitis in albino rats. Biomed Pharmacother 2017;88:658-66.
15. Akinyemi O, Iyebor EW, Osadebe CO, Oniroko NS. Proximate and phytochemical compositions of *Ricinus communis* in Ibadan, South-Western Nigeria. Am J Food Sci Nutr Res 2016;3:96-101.
16. Kamisan FH, Yahya F, Mamat SS, Kamarolzaman MF, Mohtarrudin N, Kek TL, *et al*. Effect of methanol extract of *Dicranopteris linearis* against carbon tetrachloride-induced acute liver injury in rats. BMC Complement Altern Med 2014;14:123.
17. Baker FI, Silverton RE, Pallister CJ. Baker and Silverton's Introduction to Medical Laboratory Technology. 7th ed. Nigeria: Bounty Press Ltd.; 1998. p. 339-73.
18. Reitman S, Frankel SE. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
19. Jamshidzadeh A, Fereidooni F, Salehi Z, Niknahad H. Hepatoprotective activity of *Gundelia tourenfortii*. J Ethanopharmacol 2005;101:233-7.
20. Erhirhie EO, Ilodigwe EE, Ajaghaku DL, Mbagwu IS, Moke EG. Toxicity evaluation of a commercial herbal preparation commonly used in Nigeria. Eur J Med Plants 2015;5:176-90.
21. Deniz GY, Laloglu E, Koc K, Geyikoglu F. Hepatoprotective potential of *Ferula communis* extract for carbon tetrachloride induced hepatotoxicity and oxidative damage in rats. Biotech Histochem 2019;94:334-40.
22. Anokwuru CP, Anyasor GN, Ajibaye O, Fakoya O, Okebugwu P. Effect of fractioning solvents on phenolic, flavonoid and antioxidant activities of three Nigerian medicinal plants. Nat Sci 2011;9:53-61.
23. Thouri A, Chahdoura H, El-Arem A, Hichri AO, Hassin RB. Effect of solvent extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med 2017;17:248-58.